

beating synchronously in insects with “asynchronous” IFM. However, signals that trigger SA were not detected in the tropomyosin reflection even with this time resolution.

## 62-Plat

### Structural Changes in Isometrically Contracting Insect Flight Muscle Trapped Following a Mechanical Transient

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The structural response of isometrically contracting insect flight muscle (IFM) to rapid length-step transients was analyzed by applying multivariate data analysis to 38.7 nm repeating subvolumes (repeats) in electron tomograms of quick frozen fibers that were mechanically monitored, rapidly frozen by slamming against a liquid helium cooled copper block, freeze-substituted, sectioned and stained. IFM fibers were frozen 5.5 ms after a step stretch of 6 nm/half-sarcomere in 2 ms. In the step release experiment fibers were frozen 6.5 ms following a release of 9 nm/half-sarcomere in 2.5 ms. Tomograms sampled thin sections cut  $\leq 6 \mu\text{m}$  below impact surface, recovering 1157 repeats from stretched fibers and 782 repeats from released fibers. Resolution of the actin helix and the stagger of troponin densities in the thin filament facilitated fitting a quasiatomic thin filament model independent of myosin positions, allowing objective recognition whether modeled cross-bridges were weak- or strong-binding. Strong myosin attachments are largely restricted to four actin subunits midway between successive troponin complexes, with a single exception in quick-stretched fibers. Significant changes in the types, distribution and structure of actin-myosin attachments were observed. Prepowerstroke, weak myosin attachments in the target zone are greatly reduced after the transient. However, myosin contacts with tropomyosin in and immediately M-ward of the target zone remain and are more frequent after a release. Weak attachments outside of the target zone remain relatively constant indicating a constant rate for formation of non-productive collision complexes. Following a stretch, there is an increase in the proportion of 2-headed cross-bridges. Myosin contacts with troponin are greatest after a release, and are reduced in frequency following a stretch. The results are interpreted in terms of the shortening cycle of stretch activated IFM. Supported by NIGMS and NIAMS.

## 63-Plat

### Frequency of Maximal Power Output at in vivo Myofilament Lattice Spacing Matches Drosophila Wing Beat Frequency

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<sup>1</sup>University of Vermont, Burlington, VT, USA, <sup>2</sup>University of Toronto, Toronto, ON, Canada, <sup>3</sup>Illinois Institute of Technology, Chicago, IL, USA. In striated muscle, actomyosin cross-bridge behavior is dependent on the distance between thick and thin filaments. Demembrated (skinned) fibers are often used to probe cross-bridge cycling kinetics, where the filament lattice is typically swollen from *in vivo* due to plasma membrane removal. To investigate the functional consequences of returning lattice spacing to *in vivo* values in skinned, indirect flight muscle (IFM) fibers we added a large, neutral, long-chain polysaccharide (4% w/v Dextran T-500, 500 kDa) to the bathing solution. X-ray diffraction measurements of living *Drosophila melanogaster* and skinned IFM fibers allowed us to measure *in vivo* and control for *in vitro* lattice spacing values. Small amplitude sinusoidal length perturbation analysis measured frequencies of maximal oscillatory work ( $112 \pm 3$  Hz) and power ( $145 \pm 5$  Hz) at *in vivo* lattice spacing ( $15^\circ\text{C}$ ). This suggests that *in vivo* cross-bridge kinetics are tuned for power output, as the wing beat frequency of fruit flies is  $\sim 150$  Hz at  $15^\circ\text{C}$ . To confirm these shifts in cross-bridge kinetics arise from changes in lattice spacing rather than osmotic pressure, we matched the osmotic pressure of 4% T-500 using 0.34% Dextran T-10 (10 kDa), a shorter polysaccharide that minimally affects filament lattice spacing at this concentration. Work and power were unchanged at 0.34% T-10, compared to measurements without dextran. Importantly, the frequencies of maximal work and power output were  $143 \pm 6$  and  $202 \pm 4$  Hz in a swollen lattice without dextran, demonstrating a significant difference from *in vivo* spacing. These results signify that *in vivo* lattice spacing optimizes cross-bridge cycling kinetics for power output, not work, during flight.

## 64-Plat

### Force Enhancement in the Drosophila Jump Muscle

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Force Enhancement (FE), a history-dependent phenomenon observed in skeletal muscle, is characterized by an elevated steady-state force after an active stretch compared to that of an isometric contraction at the corresponding length. FE has been observed for nearly a century, and demonstrated in whole muscle and single fiber preparations. Although the underlying mechanism(s) are yet to be elucidated, FE has been well characterized on both the ascending and descending limb of the force-length relationship, demonstrating a positive correlation with stretching amplitude and no clear relation to stretching rate. Furthermore, evidence has suggested that the mechanism for FE is multi-fold, encompassing both a passive structural element (titin) and kinetic mechanism. The limitations of investigating FE arise from the ability to manipulate molecular structure of the sarcomere in skeletal muscle. For that reason, it is advantageous to study alternative models. We have recently demonstrated the *Drosophila*'s Tergal Depressor of the Trochantor (TDT), or jump muscle, to be an analogous to that of skeletal muscle. Furthermore, our ability to genetically manipulate the structure and subsequent myosin kinetics of the TDT muscle through transgene expression allows unprecedented insight into an analogous muscle model. Therefore, the TDT offers unique opportunities to investigate the underlying mechanism(s) associated with FE. The purpose of this investigation was to confirm the presence of FE in a wild-type (WT) *Drosophila* TDTs. TDT muscles were dissected, prepared, and mechanically evaluated on a custom-built, microscope-based mechanics rig as previously established. Preliminary results not only suggest that FE exists in WT TDTs, but that this phenomenon is also characteristically similar to that observed in mammalian skeletal muscle.

## 65-Plat

### A model with Heterogeneous Half-Sarcomeres Exhibits Residual Force Enhancement After Active Stretch

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A skeletal muscle fiber that is activated and then stretched from L1 to L2 produces more force after the transient decays than if it was activated at L2. This behavior has been well studied experimentally, and is known as residual force enhancement. The underlying mechanism remains controversial. We hypothesized that residual force enhancement could reflect mechanical interactions between heterogeneous half-sarcomeres.

To test this hypothesis, we subjected a computational model of interacting heterogeneous half-sarcomeres [Campbell KS, PLoS Comput Biol 2009;5(11)] to the same activation and stretch protocols that produce residual force enhancement in real preparations. Following a transient period of elevated force associated with active stretching, the model predicted a slowly decaying force enhancement lasting  $>30$  seconds after stretch. Enhancement was on the order of 10% above isometric tension at the post-stretch muscle length, which agrees closely with experiments under similar conditions. Force enhancement in the model was proportional to stretch magnitude but did not depend strongly on the velocity of stretch, also in agreement with experiments. Small but noticeable enhancement could be seen at muscle lengths on the ascending limb of the static length-tension curve, and increased with length up to an average half-sarcomere length of  $\sim 1400$  nm. Even small variability in the strength of half-sarcomeres (5% standard deviation, normally distributed) was sufficient to produce a 5.7% force enhancement over isometric tension. Model analysis suggests that heterogeneity in half-sarcomeres leads to residual force enhancement by storing strain energy introduced during active stretch in crossbridge populations and in passive structures within and between half-sarcomeres. Complex interactions between the heterogeneous half-sarcomeres then dissipate stored energy at a rate much slower than that of crossbridge cycling, thereby producing force enhancement.

## PLATFORM D: Intrinsically Disordered Proteins I

## 66-Plat

### Charge Interactions Can Dominate the Dimensions of Intrinsically Disordered Proteins

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